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KS 1529 (2012) (English): Pomades and solid
brilliantines – Specification (Draft Standard)



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**Pomades and solid brilliantines —
Specification**

PUBLIC REVIEW DRAFT

KS 1529: 2012

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Pomades and solid brilliantines — Specification

PUBLIC REVIEW DRAFT

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Foreword

This third Edition of this Kenya Standard was prepared by the Technical Committee on the Cosmetics and related products, under the guidance of Standards Projects Committee and it is in accordance with the procedures of the Bureau.

Pomades and brilliantines are products with a wide application area, though they are mainly used for hair and skin care. A limit for heavy metals is therefore given for safety purposes. Other requirements specified include melting point, sulphated ash, penetration cone, rancidity, bleed number, stability, and microbiological examination.

This standard only covers pomades and solid brilliantines. Liquid brilliantines are covered in the specification for hair oils.

This standard was first issued in the year 2000. In this second edition of 2004, a provision was made for products containing sulphur for control of itchy scalp. Also, methods of test for lead arsenic and mercury were replaced with current ones. In addition, testing of specific pathogenic micro-organisms was introduced, and the marking clause was altered. Also, the scope was widened to cover pomades or solid brilliantines containing insect repellents.

During the preparation of this standard, reference was made to the following documents:

IS 9339-1988: Specification for pomades and brilliantines.

Regulation (EC) No. 1223/2009 of the European Parliament and of the Council of the European Union. (2009): **Cosmetic Products**. Brussels.

Acknowledgement is hereby made for the assistance derived from these sources.

Pomades and solid brilliantines — Specification

1 Scope

1.1 This Kenya Standard prescribes requirements and methods of test for pomades and solid brilliantines.

1.2 This standard applies to:

- i) pomades and solid brilliantines based on vegetable oils, mineral oils, animal fats, waxes, or fatty acids but excludes oil emulsions.
- ii) pomades and solid brilliantines to which sulphur has been added as an intrinsic part of the formulation.
- iii) insect repellents manufactured in the form of pomades or solid brilliantines.

1.3 This standard does not cover:

- i) liquid brilliantines. These are covered in KS 03-602*.
- ii) pomades and brilliantines which contain ingredients that affect the physiological functions of the body, scalp or hair, or for which therapeutic claims are made. such products shall be registered by the Ministry responsible for registration of medicated products

2 Normative references

The following standards are necessary adjuncts to this standard. They contain provisions which through reference in this text constitute provisions of this standard.

| | |
|---------|---|
| KS 1474 | <i>Classification of cosmetic raw materials and adjuncts.</i> |
| KS 1707 | <i>Labelling of cosmetic products.</i> |
| KS 1668 | <i>Methods of sampling cosmetics.</i> |

3 Definitions

For the purposes of this standard, the following definitions shall apply:

3.1

solid brilliantine

a hair dressing preparation based on petroleum jelly. May contain dyes and perfumes

3.2

liquid brilliantine

liquid mineral, vegetable or animal oil for body and hair care

3.3

pomade

an ointment used for cosmetic purposes. May be greasy, soft or hard, perfumed or non-perfumed. May contain mineral oil, vegetable oil, animal fat, or a combination of the three

3.4

petroleum jelly

a mixture of petroleum hydrocarbons used as an emollient. Usually consists of mineral oil and waxes like microcrystalline wax

* Specification for hair oils.

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4 Requirements

4.1 Visual appearance

The pomade and solid brilliantine shall be in the form of a soft, homogenous unctuous mass.

4.2 Ingredients

4.2.1 All raw materials used in the manufacture of pomades and solid brilliantines shall comply with all parts of KS 1474. In addition, the mineral oils, vegetable oils, animal fats and waxes shall comply with the relevant Kenya standards, wherever available (see Annex P).

4.2.2 All products containing ingredients that affect the physiological functions of the body, scalp or hair, or medicinal products for which therapeutic or medical claims are made shall be registered with the Ministry of Health.

4.2.3 All essential oils/herbs used shall comply with the approved standards where such exist.

4.2.4 All products containing herbal extracts known to have antibacterial activity e.g. neem oil/extract, and aloe oil/extract, shall pass the test for antibacterial and antifungal activity outlined in Annex Q. Further details may also be obtained from Annex N. All products claiming antibacterial effects shall be also be tested for antibacterial activity.

4.2.5 All insect repellents manufactured in the form of pomades or solid brilliantines shall be registered with the Pest Control Products Board, which shall determine their efficacy before issuing a licence.

4.2.6 All ingredients used shall be in sufficient quantities to effect the claims made.

4.2.7 The raw materials used shall be free from any harmful effect on the skin, scalp or hair and the interaction of the raw materials used in the finished pomade or brilliantine shall be free from toxic or sensitizing effects.

4.2.8 A list of ingredients conventionally used in the formulation of pomades and brilliantines of various types is given for information only in Annex P.

4.2.9 Use of sulphur as an ingredient

4.2.9.1 Sulphur may be added to products as an ingredient to form an intrinsic part of the formulation.

4.2.9.2 The main purpose of the product shall be to sooth itchy scalp.

4.2.9.3 Such a product shall fulfil the following conditions:

- i) shall contain a maximum of 3 % sulphur (w/w) when tested in accordance with Annex A (see Table 1);
- ii) shall be clearly labelled as per 7.1 and 7.2;

4.2.9.4 All other products in which sulphur is not added as an intrinsic part of the formulation shall not contain sulphur or sulphides when tested in accordance with Annex B (see Table 1).

4.3 Other requirements

All pomades and brilliantines shall also comply with the requirements given in Tables 2, 3 and 4 when tested according to the methods prescribed therein.

Table 1 — Sulphur and sulphide limits

| SL No. | Characteristic | Requirement | | Test method |
|--------|-----------------------|--|--------------------------|-------------|
| | | Products containing sulphur as an ingredient | Products without sulphur | |
| i) | Total sulphur content | 3 % max. (m/m) | N/A | Annex A |
| ii) | Sulphur and sulphides | N/A | To pass the test | Annex B |

Table 2 — Contaminants

| SL No. | Characteristic | Limits | Test method |
|--------|---|--------|-------------|
| i) | Sulphated ash, % by mass, max ^{a)} . | 0.10 | Annex C |
| ii) | Lead, parts per million, max. | 20 | Annex D |
| iii) | Arsenic, parts per million, max. | 2 | Annex E |
| iv) | Mercury, parts per million, max. | 2 | Annex F |
| v) | Total amount of heavy metals, ppm, max. | 20 | Annex G |

^{a)} For products containing sulphur for soothing of the scalp, refer to 4.2.5.

Table 3 — Requirements for pomades and solid brilliantines

| SL No. | Characteristic | Requirements | Test method |
|--------|----------------------|------------------------------|-------------|
| i) | Melting point, °C | 35 to 60 | Annex H |
| ii) | Consistency, 1/10 mm | 100 to 275 | Annex J |
| iii) | Test for rancidity | Shall be free from rancidity | Annex K |
| iv) | Bleed number | 5 to 15 | Annex L |
| v) | Stability | To pass the test | Annex M |

Table 4 — Microbiological examination

| SL No. | Characteristic | Limits | Test method |
|--------|---|---|-------------|
| i) | <i>Microbiological examination</i> Total viable count for aerobic mesophilic micro-organisms per g, max. | 100 | Annex N |
| ii) | <i>Pseudomonas aeruginosa</i>) <i>Staphylococcus aureus</i>) <i>Candida albicans</i>) | Not detectable in 0.5 g of cosmetic product | Annex N |

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5 Packaging

The product shall be packed in suitable, airtight containers.

6 Marking and labelling

6.1 Labelling shall be as per the requirements outlined in KS 1707¹.

6.2 In addition to the above, all products containing sulphur as part of the formulation (see 4.2.5) shall be marked with the following warnings:

“Contains Sulphur”

“If irritation develops, discontinue use”

7 Sampling

Representative unopened samples shall be drawn for test from the market, factory or anywhere else following the procedures outlined in KS 1668.

The samples shall be declared as complying with the specification if they satisfy all the specified requirements.

8 Methods of test

Unless otherwise stated, analytical grade chemicals and distilled or de-ionized water shall be used in tests. All calculations shall be to 3 decimal places.

¹ KS 1707: labeling of cosmetic products

Annex A
(normative)**Testing of total sulphur content in pomades****A.1 Procedure**

Transfer about 0.5 g of sulphur ointment, accurately weighed, to a suitable conical flask. Add 5 mL of nitric acid and 3 mL of bromine. Warm slightly and allow to stand overnight. Heat gently on a steam bath until the excess bromine has been dissipated. Add 30 mL of water and then 30 mL of ether, and swirl to dissolve most of the ointment base. Transfer the mixture to a separator, with the aid of a 20-mL and a 10-mL portion of ether, followed by two 10-mL portions of water. Shake the mixture, and draw off the water layer into a suitable beaker or flask. Extract the ether layer with two 40-mL portions of water each containing 1 mL of hydrochloric acid. Heat the combined water extracts on a steam bath to remove all traces of ether. Dilute the solution with water to about 200 mL and heat to boiling. Add slowly, with constant stirring, about 20 mL of hot barium chloride TS. Heat on a steam bath for 1 hour, and then collect the precipitate on a filter. Wash it well with hot water, dry, and ignite to constant weight.

A.2 Results

The weight of the barium sulphate so obtained, multiplied by 0.1374, represents the weight of sulphur.

Test for sulphur and sulphides

B.1 Apparatus

B.1.1 Copper strips, 1 cm in width, and freshly polished.

B.2 Procedure

Melt in a beaker about 100 g of the sample and keep in a water bath at a temperature of 95 °C. Then place a strip of copper in the melted sample so that it is partially immersed in it and allow to remain for 10 minutes.

B.3 Results

The material shall be taken to have passed the test if the copper strip used in the test shows no tarnishing when compared with another freshly polished copper strip.

Annex C

(normative)

Determination of sulphated ash**C.1 Reagents****C.1.1 Dilute sulphuric acid**, approximately 5N.**C.2 Procedure**

Heat a porcelain or silica dish of 50 mL to 100 mL capacity to redness; cool in a desiccator and weigh. Place about 20 g of the sample, accurately weighed, in the dish. Heat the dish gently by means of a bunsen burner until the oil can be ignited at the surface. Remove the burner and allow the oil to burn completely, taking care that all the free carbon on the sides of the dish is completely burnt. Heat the residue with a strong flame or in a muffle furnace until all the carbonaceous matter has disappeared. Cool the dish; add a few drops of dilute sulphuric acid; heat gently to drive off the acid and then heat strongly. Cool the dish again in the desiccator and weigh it. Repeat the heating, cooling and weighing until constant mass is obtained.

C.3 Calculation

$$\text{Sulphated ash, \%, by mass} = \frac{M_1 \times 100}{M_2}$$

where

 M_1 = mass in g of the residue; and M_2 = mass in g of the sample taken for the test.

Annex D

(normative)

Determination of lead content in pomades and solid brilliantines by graphite furnace atomic absorption spectrophotometer (AAS)

D.1 Scope

This test method specifies an electrothermal atomization technique by using graphite furnace AAS method for the determination of lead content of pomades and solid brilliantines.

D.2 Warning and safety

The acids used in the test are highly corrosive and shall be handled with maximum care and where appropriate, use a fume hood during preparation of standards. Lead is very toxic/carcinogenic and must be handled with maximum care avoiding physical contact.

If spillage occurs, use adequate amounts of water and soap to wash off the spill.

D.3 Principle

Injecting of the prepared solution into a graphite furnace. Spectrometric measurements of the atomic absorption of the 228.8 spectral line emitted by lead hollow cathode lamp.

D.4 Materials

D.4.1 Reagents, chemicals and standards

D.4.1.1 Nitric acid, ρ about 1.4 g/mL.

D.4.1.2 Nitric acid (1+1) v/v

Mix 1 volume of conc. HNO_3 with 1 volume of distilled water.

D.4.1.3 Nitric acid (0.1M)

Place 17 mL of concentrated acid in 100 mL volumetric flask then top to the mark with distilled water and mix.

D.4.1.4 Lead standard solution , 1 000 ppm

In 1 litre volumetric flask, dissolve 1.598 g of $\text{Pb}(\text{NO}_3)_2$ in minimum volume of 1% v/v HNO_3 and finally top the mark using 1% HNO_3 .

NOTE Commercial grade standards can also be used when available.

D.4.1.5 Lead standard solution , 100 ppb

This shall be prepared freshly by serial dilution of the lead solution (D.4.1.4).

D.4.1.6 Purge gas , argon

Sufficiently pure, free from water and oil and free from lead.

D.4.2 Apparatus and equipment

D.4.2.1 Atomic absorption spectrometer fitted with graphite furnace

The atomic absorption spectrometer used will be satisfactory if after optimization according to the manufacturer's instructions and when in reasonable agreement with the values given by the manufacturer and it meets the performance criteria as set out in the manual.

D.4.2.2 Lead hollow cathode lamp

D.4.2.3 Ordinary laboratory apparatus

NOTE All glassware shall first be washed in hydrochloric acid (ρ about 1.19 g/mL, diluted).

D.5 Performance

D.5.1 Sample preparation

Ignite 1 g of sample at 500 ± 2 °C to ash. Extract the lead from the ash with 20 mL of 2 N HNO_3 , and repeat with 10 mL of 2 N HNO_3 . Combine the extracts and dilute to 50 mL with 0.5 N HNO_3 .

D.5.2 Calibration

D.5.2.1 Preparation of calibration curve

D.5.2.1.1 Dilute the stock 100 ppb solution with 0.1 M HNO_3 to obtain solutions with 10 ppb, 20 ppb, 40 ppb, 60 ppb, 80 ppb and 90 ppb of lead.

D.5.2.1.2 Inject 20 microlitres each of the six solutions in turns at the same rate starting from the lowest concentrated solution to the highest concentrated solution.

D.5.2.1.3 Record the corresponding absorbance values and plot calibration curve.

D.5.3 Quality control checks

D.5.3.1 Duplicates

D.5.3.1.1 All samples will be analyzed in duplicates and the stated acceptance criteria shall apply: The absolute difference between two independent test results obtained using the same procedure shall be not greater than 10 % of the arithmetic mean of the two results.

D.5.3.1.2 Spike distilled water with 10.0 ppb of lead and obtain the recovery percentage.

D.5.3.1.3 Recovery % ≥ 96 .

D.6 Procedure

D.6.1 Test portion

Use sample as prepared in (D.5.1).

D.6.2 Blank test

D.6.2.1 Run a parallel reagent blank determination replacing the test solution with distilled water.

D.6.2.2 Reagent blank shall be ≤ 0.0001 ppb of lead.

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D.6.3 Instrumentation

D.6.3.1 Follow the manufacturer's instructions for preparing the instrument for use.

D.6.3.2 Install the appropriate lamp and adjust the current to the recommended value.

D.6.3.3 Ensure that the autosampler pipette is correctly aligned and that the drain is available.

D.6.3.4 Select the sample tray type installed.

D.6.3.5 Ensure that the graphite tube is in good condition and correctly aligned.

D.6.3.6 Switch on the cooling system, turn on the purge gas and finally start the instrument software.

D.6.3.7 Select the relevant method and then condition tube.

D.6.4 Instrument conditions

The following conditions shall be used for the furnace during analysis of lead:

D.6.4.1 Wavelength: 283.3

D.6.4.2 Slit: 0.7

D.6.4.3 Atomization site: pyro/platform

D.6.4.3 The furnace conditions for lead are given in Table D.1.

Table D.1 — Furnace conditions for lead

| Step | Temperature °C | Ramp time (seconds) | Hold time (seconds) | Internal gas flow (L/min.) | Gas type |
|------------------|----------------|---------------------|---------------------|----------------------------|----------|
| 1 (drying) | 120 | 10 | 60 | 250 | Normal |
| 2 (pretreatment) | 500 | 1 | 30 | 250 | „ |
| 3 (cooling) | 20 | 1 | 15 | 250 | „ |
| 4 (atomization) | 1 800 | 0 | 5 | 0 | „ |
| 5 (cleanout) | 2 600 | 1 | 5 | 250 | „ |

D.6.5 Spectrometric measurements

D.6.5.1 Inject 20 µl of the calibration standards, the blank solution and the test solution into the graphite furnace.

D.6.5.2 Record the absorbance reading.

D.6.5.3 If the absorbance of the sample is greater than the highest calibration standard, dilute the test solution appropriately using 0.1 M HNO₃ for lead then measure the absorbance.

D.6.5.4 Inject the calibration solutions in ascending order.

NOTE 1 The calibration curve shall only be acceptable for analysis when the correlation coefficient (r) ≥ 0.99 .

D.7 Expression of results**D.7.1 Method of calculation**

The lead content of the sample expressed in mg/L of product is equal to:

$$[(C_1 - C_2) * V] / M_0$$

where

C_1 = lead content of test solution expressed in mg/L read from calibration curve;

C_2 = lead content of blank solution expressed in mg/L read from calibration curve;

M_0 = grams of sample taken for analysis (5 g);

V = Volume of sample taken for analysis (100 mL).

NOTE If the test solution was diluted, then the dilution factor shall be taken into consideration in calculation.

D.7.2 Expression of results

D.7.2.1 Report results of manganese content in mg/L as Pb in the sample into two decimal points.

D.8 Method validation

D.8.1 The method validation data shall be as shown in Table D.2.

Table D.2 — Method validation data

| Element | Linearity | LOQ ppb | LOD ppb |
|---------|---------------|---------|---------|
| Pb | $r \geq 0.99$ | 32.356 | 3.804 |

D.8.2 Precision: Repeatability

The absolute difference between two independent test results obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time shall not be greater than 10 % of the arithmetic mean of the two results.

D.8.3 Working range

Pb 10-100 ppb.

D.8.4 Reporting limits

Pb 30 ppb.

Annex E

(normative)

Test for arsenic using atomic absorption spectrophotometer (AAS)

E.1 Scope

This method prescribes the determination of arsenic in pomades and solid brilliantines.

E.2 Reagents

E.2.1 0.15 mol/L (\cong 1.5 % V/V) hydrochloric acid

Carefully add 15 mL conc. HCl to deionized water and make up to 1 L.

E.2.2 0.25 mol/L (\cong 1 % W/V) NaOH solution

Carefully dissolve 10 g sodium hydroxide flakes in deionized water and make up to 1 L.

E.2.3 0.8 mol/L (\cong 3 % W/V) NaBH₄ solution

Dissolve 3 g sodium tetrahydroborate in 1 % NaOH solution and make up to 100 mL with 1 % NaOH solution.

E.3 Stock solution

The stock solution contains 1 000 mg/L As. The use of commercially available concentrated calibration solutions for AAS is recommended.

WARNING: Arsenic solutions are toxic.

E.4 Calibration solution

1 mg As/L (in 1.5 % HCl).

E.4.1 Aliquots for calibration: 10, 25, 50 μ L

E.4.2 Corresponding to: 10, 25, 50 ng As

E.4.3 Diluent: 1.5 % (V/V) hydrochloric acid

E.4.4 Calibration volume: 10 mL

E.5 Reductant solution 3 % NaBH₄ in 1 % NaOH solution

E.6 Oxidation state

The hydride is generated much more slowly from As(V) than from As(III). To prevent interferences, As(V) must be prereduced to As(III) prior to the determination.

Prereduction can be performed with KI in semi concentrated (5 mol/L) HCl solution or, preferably, with L-cysteine

E.7 Pre-reduction**E.7.1 KI solution**

Dissolve 3 g KI and 5 g L (+)- ascorbic acid in 100 mL water. Prepare fresh daily. Add 1 mL of the KI solution per 10 mL of the sample solution in 5 mol/L HCl and stand for 30 min.

Or,

E.7.2 L-cysteine solution: Dissolve 5 g L-cysteine in 100 mL 0.5 mol/L HCl. This solution is stable for at least a month. Add 2 mL of the L-cysteine solution per 10 mL of the sample solution and stand for 30 min.

E.8 Instrument conditions

- E.8.1** Analytical wavelength: 193.7 nm
- E.8.2** Slit width and height: 0.7 nm low
- E.8.3** Radiation source: Electrodeless discharge lamp for As
- E.8.4** QTA heating: Heat the QTA in a lean, blue air-acetylene flame
- E.8.5** Prepared measurement volume: 10 mL minimum to 50 mL maximum
- E.8.6** Pre-reaction purge time: Approx. 50 s
- E.8.7** Post-reaction purge time: Approx. 40 s
- E.8.8** Characteristic mass: 0.95 ng As for 1 % absorption ($A = 0.0044$)
- E.8.9** Characteristic concentration: 0.095 $\mu\text{g/L}$ 1 % absorption for 10 mL calibration volume
- E.8.10** Characteristic concentration check: 50 μL of the 1 000 mg/L As stock solution (50 ng) give an absorbance of approx. $A = 0.2$.
- E.8.11** Alternate analytical wavelengths are given in Table E.1:

Table E.1 — Alternate analytical wavelengths

| Wavelength nm | Slit width nm | Sensitivity relative to main analytical wavelength |
|------------------|------------------|---|
| 189.0 | 0.7 | 0.8 |
| 197.2 | 0.7 | 2.0 |

NOTE Condition the QTA in cold hydrofluoric acid if there is a decrease in sensitivity (and other causes are excluded).

Annex F

(normative)

Test for mercury using atomic absorption spectrophotometer (AAS)

F.1 Method 1 — Using sodium tetrahydroborate (NaBH₄) as reductant

F.1.1 Scope

This method prescribes the determination of mercury in pomades and solid brilliantines using sodium tetrahydroborate (NaBH₄) as reductant.

F.1.2 Reagents

F.1.2.1 0.15 mol/L (≅ 1.5 % V/V) hydrochloric acid

Carefully add 15 mL conc. HCl to deionized water and make up to 1 L.

F.1.2.2 0.22 mol/L (≅ 1.5 % V/V) nitric acid

Carefully add 15 mL conc. HNO₃ to deionized water and make up to 1 L.

F.1.2.3 5 % (W/V) KMnO₄ solution

Dissolve 5 g potassium permanganate in deionized water and make up to 100 mL.

F.1.2.4 0.25 mol/L (≅ 1 % W/V) NaOH solution

Carefully dissolve 10 g sodium hydroxide flakes in deionized water and make up to 1 L.

F.1.2.5 0.8 mol/L (≅ 3 % W/V) NaBH₄ solution

Dissolve 3 g sodium tetrahydroborate in 1% NaOH solution and make up to 100 mL with 1% NaOH solution.

F.1.3 Stock solution

The stock solution contains 1 000 mg/L Hg. The use of commercially available concentrated calibration solutions for AAS is recommended.

WARNING Mercury solutions are toxic.

F.1.4 Calibration solution

1 mg Hg/L (in 1.5 % HNO₃ stabilized by the addition of a few drops of 5 % KMnO₄ solution).

F.1.4.1 Aliquots for calibration: 100, 200, 500 µL

F.1.4.2 Corresponding to: 100, 200, 500 ng Hg

F.1.4.3 Diluent: 1.5 % (V/V) nitric acid or 1.5 % (V/V) hydrochloric acid

F.1.4.4 Calibration volume: 10 mL

F.1.5 Reductant solution: 3 % NaBH₄ in 1 % NaOH solution

F.1.6 Instrument conditions

- F.1.6.1** Analytical wavelength: 253.6 nm
- F.1.6.2** Slit width and height: 0.7 nm low
- F.1.6.3** Radiation source: Electrodeless discharge lamp or hollow cathode lamp for Hg
- F.1.6.4** QTA heating: No flame required. If condensation in the QTA is a problem, heat the QTA gently by mounting an infrared lamp above it
- F.1.6.5** Prepared measurement volume: 10 mL minimum to 50 mL maximum
- F.1.6.6** Pre-reaction purge time: Approx. 5 s
- F.1.6.7** Post-reaction purge time: Approx. 50 s
- F.1.6.8** Characteristic mass: 4.68 ng Hg for 1 % absorption ($A = 0.0044$)
- F.1.6.9** Characteristic concentration: 0.468 $\mu\text{g/L}$ 1 % absorption for 10 mL calibration volume
- F.1.6.10** Characteristic concentration check: 250 μL of the 1000 mg/L Hg stock solution (250 ng) give an absorbance of approx. $A = 0.2$.

F.1.7 Notes

- F.1.7.1** Stabilize stock and calibration solutions by adding KMnO_4 or KI solution.
- F.1.7.2** Stabilize all solutions in the reaction flask by adding 1 drop of 5 % (w/v) KMnO_4 solution before starting the determination.

F.2 Method 2 — Using tin (II) chloride (SnCl_2) as reductant**F.2.1 Scope**

This method describes the determination of mercury in pomades and solid brilliantines using tin (II) chloride (SnCl_2) as reductant.

F.2.2 Reagents**F.2.2.1 0.15 mol/L (\cong 1.5 % V/V) hydrochloric acid**

Carefully add 15 mL conc. HCl to deionized water and make up to 1 L.

F.2.2.2 1 mol/L (\cong 10 % V/V) hydrochloric acid

Carefully add 100 mL conc. HCl to deionized water and make up to 1 L.

F.2.2.3 0.22 mol/L (\cong 1.5 % V/V) nitric acid

Carefully add 15 mL conc. HNO_3 to deionized water and make up to 1 L.

F.2.2.4 5% (W/V) KMnO_4 solution

Dissolve 5 g potassium permanganate in deionized water and make up to 100 mL.

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F.2.2.5 5 % (W/V) SnCl₂

Dissolve 50 g tin (II) chloride dihydrate (SnCl₂ · 2H₂O) in 10 % HCl solution and make up to 1 L with 10 % HCl solution.

F.2.3 Stock solution

The stock solution contains 1 000 mg/L Hg. The use of commercially available concentrated calibration solutions for AAS recommended.

WARNING: Mercury solutions are toxic.

F.2.4 Calibration solution

1 mg Hg/L (in 1.5 % HNO₃ stabilized by the addition of a few drops of 5 % KMnO₄ solution).

F.2.4.1 Aliquots for calibration: 100, 200, 500 µL

F.2.4.2 Corresponding to: 100, 200, 500 ng Hg

F.2.4.3 Diluent: 5 % (V/V) nitric acid or 1.5% (V/V) hydrochloric acid

F.2.4.4 Calibration volume: 10 mL

F.2.5 Reductant solution 5 % SnCl₂ · 2H₂O in 10 % HCl solution.

F.2.6 Instrument Conditions

F.2.6.1 Analytical wavelength: 253.6 nm

F.2.6.2 Slit width and height: 0.7 nm low

F.2.6.3 Radiation source: electrodeless discharge lamp or hollow cathode lamp for Hg

F.2.6.4 QTA heating: no flame required. If condensation in the QTA is a problem, heat the QTA gently by mounting an infrared lamp above it.

F.2.6.5 Prepared measurement volume: 10 mL minimum to 50 mL maximum.

F.2.6.6 Pre-reaction purge time: Approx. 5 s

F.2.6.7 Post-reaction purge time: Approx. 50 s

F.2.6.8 Characteristic mass : 4.68 ng Hg for 1 % absorption (A = 0.0044).

F.2.6.9 Characteristic concentration: 0.468 µg / L / 1 % absorption for 10 mL calibration volume.

F.2.6.10 Characteristic concentration check: 250 µL of the 1 000 mg/L Hg stock solution (250 ng) give an absorbance of approx. A = 0.2

F.2.7 Notes

F.2.7.1 Stabilize stock and calibration solutions by adding KMnO₄ solution. Do not use KI solution since iodide interferes in the release of mercury.

F.2.7.2 Stabilize all solutions in the reaction flask by adding 1 drop of 5 % (W/V) KMnO₄ solution before starting the determination.

Annex G
(normative)

Total amount of heavy metals

The total amount of heavy metals shall be calculated by adding up the values obtained for lead (Annex D), arsenic (Annex E), and mercury (Annex F). The report shall be given in ppm.

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Annex H
(normative)

Determination of melting point

H.1 Procedure

H.1.1 Heat a quantity of the sample on a water bath while stirring until it reaches a temperature of 90 °C to 92 °C. Cool the molten sample to a temperature of 8 °C to 10 °C above the expected melting point. Chill the bulb of a thermometer (range 1 °C to 100 °C) to 5 °C, wipe it dry and while it is still cold, dip it into the molten sample so that approximately half of the bulb is submerged. Withdraw it immediately and hold it vertically away from heat until the wax surface dulls, then dip it for five minutes into a water bath having a temperature not higher than 16 °C.

H.1.2 Fix the thermometer prepared securely in a test tube so that its lowest point is about 15 mm above the bottom of the test tube. Suspend the test tube in a water bath adjusted to 16 °C and raise the temperature of the bath at a rate of 2 °C per minute up to 30 °C, then change the rate of rise to 1 °C per minute and note the temperature at which the first drop of the melted sample leaves the thermometer. Repeat the determination twice on a freshly melted portion of the sample. If the variation in three determinations is less than 1 °C take the average of the three as the melting point. If the variation in three determinations is more than 1 °C, make two additional determinations and take the average of five.

Annex J
(normative)

Determination of consistency

J.1 Determination of consistency of the material is made by measuring the penetration of a standard cone at 25.0 ± 0.5 °C.

J.1.2 Apparatus

J.1.2.1 Penetrometer

Any suitable penetrometer which permits the specified cone to drop vertically without appreciable friction for at least 40 mm and which indicates accurately the depth of penetration to the nearest 0.1 mm. The instrument shall have a table to carry the test sample which may be adjusted to the horizontal before conducting the test. A mechanism for releasing and clamping the loaded cone shall be provided.

J.1.2.2 Cone

Consisting of a conical body of brass or corrosion resistant steel with detachable hardened steel tip, constructed to conform to the dimensions and tolerances shown in Figure J.1. The total moving mass, namely, that of the cone and its movable attachments shall be 150.0 ± 0.1 g. The attachments consist of a rigid shaft having a suitable device at its lower end for engaging the cone. The outer surface shall be polished to a very smooth finish.

All dimensions in millimetres

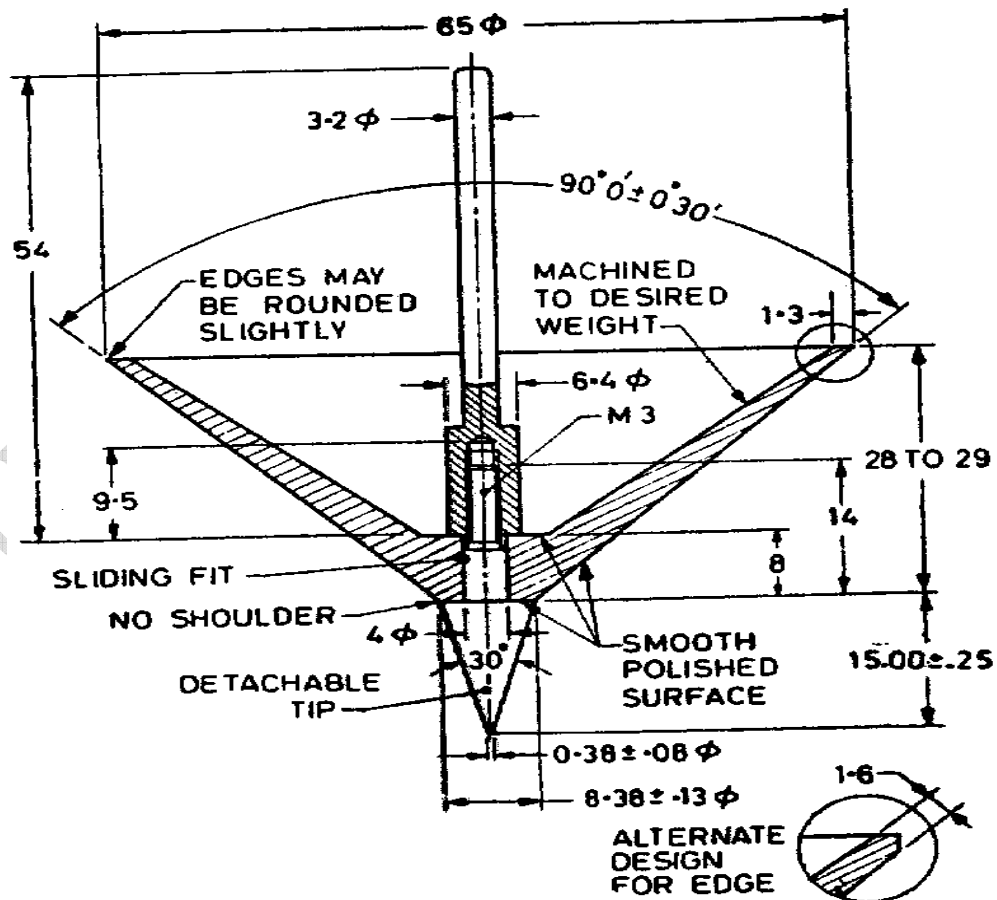


Figure J.1— Penetrometer cone

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J.1.2.3 Constant temperature bath

A water bath capable of regulating the temperature at 25.0°C and of suitable design for conveniently bringing the sample container to the test temperature. The bath shall be provided with a cover to maintain the temperature of the air above the sample at 25°C .

J.1.2.4 Timing device

A stop-watch or any other suitable instrument capable of measuring an interval of 5 seconds to an accuracy of 0.2 seconds.

J.1.2.5 Sample container

Flat-bottomed; metal or glass cylinders that are 100 ± 5 mm in diameter and not less than 60 mm in height.

J.1.3 Procedure

J.1.3.1 Melt a quantity of the sample at $82.0 \pm 2.5^{\circ}\text{C}$, pour into one or more of the sample containers, filling to within 6 mm of the brim. Cool at $25.0 \pm 0.5^{\circ}\text{C}$ over a period of not less than 16 hours, protecting from draughts. Two hours before the test; place the containers in a water bath at $25.0 \pm 0.5^{\circ}\text{C}$. If the room temperature is below 23.5°C or above 26.5°C , adjust the temperature of the cone to $25.0 \pm 0.5^{\circ}\text{C}$ by placing it in a water bath.

J.1.3.2 Without disturbing the surface of the sample, place the container on the penetrometer table, and lower the cone until the tip just touches the top surface of the sample at a spot 25 mm to 39 mm from the edge of the container. Adjust the zero setting and quickly release the plunger, then hold it free for 5 seconds. Secure the plunger, and read the total penetration from the scale. Make three or more trails each so spaced that there is no overlapping of the areas of penetration. Where the penetration exceeds 20 mm, use a separate container of the sample for each trial. Read the penetration to the nearest 0.1 mm. Calculate the average of three or more readings; and conduct further trials to a total of 10 if the individual results differ from the average by more than $\pm 3\%$.

J.1.4 Calculation

Cosistency = $10 A$

where

A = mean of all the values of penetration in mm.

Annex K
(normative)

Test for rancidity

K.1 Reagents

K.1.1 Concentrated hydrochloric acid, Analytical reagent grade.

K.1.2 Phloroglucinol solution

Dissolve 0.1 g of phloroglucinol in 100 mL of diethyl ether.

K.2 Procedure

Shake 10 mL of the material, melt if necessary, with 10 mL of concentrated hydrochloric acid and 10 mL of phloroglucinol solution: Shake for 1 minute.

J.3 Results

The material shall be taken to have passed the test if no pink colour develops.

Annex L
(normative)

Bleed number

L.1 Procedure

Heat the sample to 95 °C. Then allow to cool to 10 °C above its melting point. Dip a glass tube (or internal diameter 4 mm and wall thickness 1 mm) into the sample so that when it is removed with the upper end closed with a finger, it contains approximately 25 mm column of molten sample. From approximately 12 mm above the filter paper (Whatman No. 1 or equivalent), allow 5 evenly spaced drops of the sample to fall separately on the paper. The droplets shall have a diameter of 6 mm to 8 mm. When the droplets solidify, place the paper on a watch glass and insert in an oven kept at 30 °C for 24 hours. After 24 hours, determine the diameter of each droplet plus the oil ring which surrounds it. Subtract the diameter of the droplet from the oil ring and record the result in mm. The average of these result in mm.

Annex M
(normative)**Test for stability****M.1 Apparatus**

M.1.1 Ultra violet lamp, with emission at 360 nm.

M.2 Procedure

Place 50 mL of the material in a 100 mL glass beaker. Turn on the ultra violet lamp and expose the samples at a distance of 12 cm to 14 cm below the lamp for 6 hours. After the specified time, remove the sample, cool to room temperature and compare for any change in odour or colour. The same volume of material shall be employed for all tests so that comparison is ensured on a reproducible basis.

NOTE The output of the ultra violet lamp diminishes with time in service. A log of number of hours of the lamp in use shall be maintained. The lamp is to be replaced after the specified hours of service, as recommended by the lamp manufacturer.

M.3 Evaluation

Evaluation is done by comparing the test material against an unexposed specimen from the same sample.

Annex N
(normative)

Determination of yeasts and moulds in pomades and solid brilliantines

N.1 Introduction

N.1.1 This method is obtained from the AOAC official method 998.10 entitled

“Efficacy of preservation of non-eye area water-miscible cosmetic and toiletry formulations”.

N.1.2 Acknowledge of microbiological techniques is required for these procedures. A general aseptic and safety procedures shall be followed.

N.1.3 Table N.1 gives the results of the interlaboratory study supporting the acceptance of the method.

N.2 Principle of the method

Bacteria yeast and mould grown on laboratory media, harvested, calibrated, and inoculated into test products. Using serial dilutions and plate counts, the numbers of organisms surviving in the test products are determined over time. Products meeting the specified criteria are considered adequately preserved for manufacture and consumer use. Products not meeting criteria are considered inadequately preserved.

N.3 Apparatus

N.3.1 **Jars** –2-4 oz wide-mouth, straight-side flint glass ointment jars with linerless metal, polypropylene or teflon lined screw caps.

N.3.2 **Disposable borosilicate glass culture tubes**, 16 x 125 mm, with caps.

N.3.3 **Disposable borosilicate glass culture tubes**, 20 mm x 150 mm, with screw caps.

N.3.4 **Petri plates**, 100 mm x 15 mm.

N.3.5 **Sterile 2.2 mL pipettes**.

N.3.6 **Sterile swabs**.

N.3.7 **Glass beads**.

N.3.8 **Sterile gauze**.

N.3.9 **µL 10 to 20 µL inoculating loops**.

N.3.10 **Vortex mixer**.

Table N.1 — Interlaboratory study results for determination of preservation of non-eye area water-miscible cosmetic and toiletry formulations

| Product name | Incidence of false-negatives among total positive samples ^{a)} | | | Incidence of false-positives among total negative samples ^{b)} | | |
|---|---|------------|------------------|---|------------|------------------|
| | Number | Percentage | Sensitivity rate | Number | Percentage | Sensitivity rate |
| Shampoo | 2/49 | 4 | 96 | 0/53 | 0 | 100 |
| Conditioner | 3/48 | 6 | 94 | 0/54 | 0 | 100 |
| Water-in-oil emulsion | 0/52 | 0 | 100 | 1/50 | 2 | 98 |
| Oil-in-water emulsion | 0/51 | 0 | 100 | 0/51 | 0 | 100 |
| All combined | 5/200 | 2 | 98 | 1/208 | 0.5 | 99.5 |
| <p>^{a)} False-negative analysis indicates a sample is adequately preserved.</p> <p>^{b)} False-positive analysis indicates a sample is not adequately preserved.</p> | | | | | | |

N.4 Reagents

For convenience, dehydrated media of any brand equivalent in function may be used. Test each lot of medium for sterility and growth-promotion using suitable organisms.

N.4.1 Lethen agar

Contains 5.0 g pancreatic digest of casein 1.0 g dextrose, 3.0 g beef extract, 1.0 g lecithin, 7.0 g polysorbate 80, and 15.0 g agar per L. Prepare according to manufacturer's directions. Dispense into suitable containers and sterilize by autoclaving at 121 °C for 15 min. Final pH shall be 7.0 ± 0.2 at 25 °C. Place in 45 °C water bath until agar is 45 ± 2 °C. Use for pour plates.

N.4.2 D/E neutralizing broth (Dey/Engley)

Contains 5.0 g pancreatic digest of casein, 2.5 g yeast extract, 10 g dextrose, 1.0 g sodium thioglycollate, 6.0g Na₂ S₂O₃ · 5H₂O, 2.5 g NaHSO₃, 7.0 g lecithin, 5.0 g polysobate 80, and 0.02 g bromcresol purple per L. Prepare according to manufacturer's directions. Dispense 9 or 9.9 mL aliquot into tubes and sterilize by autoclaving at 121 °C for 15 min. Final pH shall be 7.6 ± 0.2 at 25 °C. Use for aerobic plate count, L, dilutions.

N.4.3 Nutrient agar

Contains 5.0 g pancreatic digest of gelatin 3.0 g beef extract and 15.0 g agar per L. Prepare according to manufacturer's directions. Dispense into tubes and sterilize by autoclaving at 121 °C for 15 min. Final pH shall be 6.8 ± 0.2 at 25 °C. Cool in inclined position to form a slant. Use for bacterial culture maintenance and inoculum preparation.

N.4.4 Y/M agar (yeast/malt extract)

Contains 3.0 yeast extract, 3.0 g malt extract, 5.0 g peptone. 10.0 g dextrose, and 20.0 g agar per L. Prepare according to manufacturer's directions. Dispense into tubes and sterilize by autoclaving at 121 °C for 15 min. Final pH shall be 6.2 ± 0.2 at 25 °C. Cool in slanted position. Use for yeast culture maintenance and inoculum preparation.

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N.4.5 Potato dextrose agar (PDA)

Contains 200 g potato infusion, 20.0 g dextrose, and 15.0 g agar per L. Prepare according to manufacturer's directions. Dispense into tubes and sterile by autoclaving at 121 °C for 15 min. Final pH shall be 5.6 ± 0.2 at 25 °C. Cool in slanted position. Use for mould culture maintenance and inoculum preparation.

N.4.6 0.85 % Saline

Dissolve 8.50 g NaCl in water and dilute to 1 L. Dispense into flasks or bottles and sterilize by autoclaving at 121 °C for 15 min.

N.4.7 0.85 % saline with 0.05 % polysorbate 80

Dissolve 8.5 g NaCl in water, mix in 0.50 g polysorbate 80, and dilute to 1 L. Dispense into suitable containers and sterilize by autoclaving at 121 °C for 15 min.

N.4.8 Barium sulphate standard No. 2

N.4.8.1 Prepare a 1.0 % BaCl₂ solution by dissolving 1.0 g BaCl₂ · 2H₂O in 100 mL water. Let this be referred to as solution 1.

N.4.8.2 Prepare a 1.0 % H₂SO₄ solution by mixing 1.0 mL H₂SO₄ in 100 mL water. Let this be referred to as solution 2.

N.4.8.3 Mix 0.2mL of solution 1 with 9.8 mL solution 2, in screw-capped test tube. Cap tightly and store in the dark at room temperature.

N.4.9 Barium sulphate standard No. 7

Use solutions from N.4.8. Mix 0.7 mL of solution N.4.8.1, with 9.3 mL of solution N.4.8.2, in a screw-capped test tube. Cap tightly and store in the dark at room temperature.

N.5 Micro-organisms

| | | | |
|---------------|-----------------------------------|---|------------|
| N.5.1 | <i>Staphylococcus aureus</i> | — | ATCC 6538 |
| N.5.2 | <i>Staphylococcus epidermidis</i> | — | ATCC 12228 |
| N.5.3 | <i>Klebsiella pneumoniae</i> | — | ATCC 10031 |
| N.5.4 | <i>Escherichia coli</i> | — | ATCC 8739 |
| N.5.5 | <i>Enterobacter gergoviae</i> | — | ATCC 33028 |
| N.5.6 | <i>Pseudomonas aeruginosa</i> | — | ATCC 9027 |
| N.5.7 | <i>Burkholderia cepacia</i> | — | ATCC 25416 |
| N.5.8 | <i>Acinetobacter baumannii</i> | — | ATCC 19606 |
| N.5.9 | <i>Candida albicans</i> | — | ATCC10231 |
| N.5.10 | <i>Aspergillus niger</i> | — | ATCC 16404 |

NOTE Environmental micro organisms (s) likely to be contaminants of concern during product manufacture or use be included as a separate inoculum. Predominant environmental microbes isolated during manufacturing, equipment cleaning, and sanitizing, or from related deionised water systems are used as supplemental test inocula).

N.6 Product quality check

N.6.1 Weigh 1.0 g product into a screw-capped culture tube containing 9.0 mL sterile neutralizing broth to make a 1:10 dilution. If necessary to disperse product, add ten to twenty 3 mm diameter glass beads to tube. Mix on vortex mixer until homogeneous.

N.6.2 Pipet 1.0 mL of the 1:10 dilution into each of 4 sterile petri plates. Pour 15-20 mL sterile molten Lethen agar (45 ± 2 °C) into each plate. Mix by rotating plates to disperse the dilution thoroughly. Let it solidify.

N.6.3 Invert and incubate 2 plates at 35 ± 2 °C for 48 h and 2 plates at 25 ± 2 °C for 5 days.

N.6.4 Count the number of colonies on all plates, add, and multiply by 2.5 to determine the number of colony forming units per gram (cfu./g) in the product.

N.6.5 Save plates to be used for the neutralization validation in N.14 by refrigerating.

N.7 Product preparation

N.7.1 Measure 20 mL sterile saline into 4 sterile jars, N.3.1. Cap tightly and store at room temperature.

N.7.2 Weigh 20 g product into each of 4 sterile jars, N.3.1. Cap tightly and store at room temperature.

N.8 Bacterial inocula preparation

N.8.1 Streak each bacteria culture, N.5.1 – N.5.10 onto a nutrient agar, N.4.3, slant. Incubate for 48 h at 35 ± 2 °C. Wash each slant with 5.0 mL sterile saline, loosening the culture from the agar surface. Transfer the suspension into a sterile tube. Repeat the wash with a second 5.0 mL aliquot of saline. Combine washes and mix on vortex mixer to disperse evenly.

N.8.2 Adjust each wash with sterile saline to yield a suspension of ca 10^8 cfu/mL using Mc-Farland BaSO₄ standard No. 2, N.4.8, direct microscopic count, turbidimetry, absorbance, or other method correlated to an aerobic plate count (APC), N.13. Perform an APC, N.13, on each suspension to confirm standardization.

N.9 Fungal inoculum preparation

N.9.1 Streak *C. albicans*, N.5.9, on 3 slants of Y/M agar, N.4.4. Incubate at 25 ± 2 °C for 48 h. Wash each slant sequentially with 5.0 mL aliquot of sterile saline. Repeat with a second 5.0 mL aliquot of sterile saline. Combine washes to produce 10 mL suspension. Mix on vortex mixer to disperse evenly.

N.9.2 Adjust the wash with sterile saline to yield a suspension of ca 10^7 cfu/mL using a Mc-farland BaSO₄ standard No. 7, N.4.9, direct microscopic count, turbidimetry, absorbance, or other method that has been correlated to an APC, N.13. Perform an APC, N.13, on the suspension to confirm standardization.

N.9.3 Streak *A. Niger*, N.5.10, on 5 slants of potato dextrose agar N.4.5. Incubate at 25 ± 2 °C for 10 days. Dislodge mould spores by adding 5.0 mL sterile saline containing 0.05 % polysorbate 80 to each tube and vigorously rubbing the surface of the agar slant with a sterile swab. Repeat with a second 5.0 mL aliquot in each tube. Combine the 10 washes to produce 50 mL suspension. Filter into a sterile container through 3-5 layers of sterile gauze supported in funnel. Perform an APC, N.13, using appropriate dilutions. Adjust mould suspension to ca 10^7 per mL using sterile saline. Use immediately or refrigerate at $2 - 5$ °C for up to 1 month. Verify mould viability by an APC, N.13, before each use.

N.10 Inoculum pools

N.10.1 Pool equal parts of the *S. aureus* and *S. epidermidis* suspensions, N.8.2 in a sterile container to make inoculum pool 1: gram-positive cocci.

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N.10.2 Pool equal parts of the *K. pneumoniae*, *E. coli*, and *E. gergoviae* suspensions, N.8.2, in a sterile container to make inoculum pool 2: gram — negative fermentors.

N.10.3 Pool equal parts of the *P. aeruginosa*, *B. cepacia* and *A. baumannii* suspensions, N.8.2, in a sterile container to make inoculum pool 3: gram — negative nonfermentors.

N.10.4 Pool equal parts of *C. Albicans*, N.9.2, and *A. Niger*, N.9.3, suspensions in a sterile container to make inoculum pool 4: fungi.

N.10.5 Use organism pools immediately or refrigerate them at 2 to 5 °C for more than 72 h.

N.11 Inoculation

N.11.1 Inoculate each of the four 20.0 mL aliquots of sterile saline, N.7.1, with 0.2 mL of its respective inoculum pool, N.10.1 – N.10.4. Mix thoroughly. Use these suspensions to determine inoculum counts (see K (a)).

N.11.2 Inoculate each of the four 20 g products suspensions, F (b) with 2.0 mL of its respective inoculum pool I(a) –d). Mix thoroughly by shaking, vortex mixing or stirring, so that each suspension contains 10^6 bacteria or 10^5 fungi per gram, evenly distribute throughout the product. Tightly close inoculated containers and store at ambient temperature (20 to 25 °C).

N.12 Sampling intervals

N.12.1 Sample each inoculated saline suspension, N.11.1, for APC, N.13, within 1 h after inoculation to obtain inoculum count.

N.12.2 Test each inoculated product, N.11.2 for APC, N.13 at 7, 14 and 28 days after inoculation to obtain product interval count.

N.13 Aerobic plate count (APC)

N.13.1 Mix suspension thoroughly, weigh 1.0 g product into screw-capped culture tube containing 9.0 mL sterile neutralizing broth for a 1: 10 dilution. If necessary to disperse product, add 10 – 20 sterile 3 mm diameter glass beads to the tube. Mix on vortex mixer until homogeneous.

N.13.2 Aseptically pipette 0.1 mL of the 1: 10 dilution into 9.9 mL tube of neutralizing broth to obtain a 1: 1 000 dilution. Vortex mix. Pipette 0.1 mL of the 1:1 000 dilution into 9.9 mL neutralizing broth to obtain a 1: 100 000 dilution. The number of dilutions may be decreased if previous counts microbial populations show reduction.

N.13.3 Using a 2.2 mL pipette, aseptically pipette 1.0 mL and 0.1 mL aliquots from the 1:10 dilution into duplicate petri dishes for the 1:10 and 1:100 plates. If necessary, transfer duplicate 1.0 and 0.1 mL aliquots from the 1: 1 000 dilution for plates 1:1 000 and 1: 10 000, and from the 1: 100 000 dilution for plates 1: 100 000 and 1: 1 000 000. Pour 15 – 20 mL sterile letheen agar N.4.1, at 45 ± 2 °C into each plate. Mix by rotating the plates to disperse the suspension thoroughly, and let solidify.

N.13.4 Invert bacterial plates and incubate at 35 ± 2 °C. Examine bacterial plates after 48 – 72 h. Count in suitable range (30 – 300 colonies). If no countable plates fall in that range, count the plates(s) nearest that range showing distinct colonies. Average duplicate plates counts and express results as cfu/g of product.

N.13.5 Invert and incubate fungal plates at 25 ± 2 °C. Read fungal plates at 2 – 3 days and record results. Count plates in a suitable range (30 – 300 colonies). If no countable plates fall in that range, count the plate(s) nearest that range showing distinct colonies. Reincubate plates for another 2 – 3 days. Read and record additional colonies. Add to previous results to obtain total counts. Average duplicate plate counts and record as cfu/g of products.

N.14 Neutralization check

Make a 1:10 000 dilution in sterile saline of pools 1, 2 and 3, N.10.1 – N.10.3, and a 1: 1 000 dilution of pool 4, N.10.4. Streak each dilution for isolation with a 10 µL loop on the plates saved from N.6.5. If plates are not usable due to either desiccation or surface growth, repeat N.6, and streak freshly prepared plates. Incubate as in N.13.4 and N.13.5.

N.15 Data analysis

N.15.1 Product quality check, N.6.4, must be found to contain, <100 cfu/g to proceed with the challenge test.

N.15.2 Inoculum counts, N.12.1, shall be between 1 to 9.9×10^6 cfu/g product for bacteria and 1 to 9.9×10^5 cfu/g product for fungi, or the test shall be repeated with different dilutions.

N.15.3 Neutralization check, N.14, must show significant growth of all pools to confirm adequate neutralization. A neutralizing broth other than D/E broth can be used. If neutralization does not occur, the test is invalid.

N.15.4 Calculate the percentage reduction:

$$\text{Reduction, \%} = \frac{\text{Inoculum count} - \text{product interval count}}{\text{Inoculum count}} \times 100$$

N.15.5 The test product is considered adequately preserved if:

- a) bacteria show at least 99.9 % (3 log) reduction within 1 week following challenge and remain at or below that level thereafter; and
- b) fungi show at least a 90 % (1 log) reduction within 1 week following challenge, and remain at or below that level thereafter. These criteria apply to freshly prepared formulations.

Annex P
(informative)

List of raw materials conventionally used in the formulation of pomades and brilliantines

P.1 Type 1

Based on mineral oils and waxes.

- a) paraffin wax;
- b) microcrystalline wax;
- c) petroleum jelly;
- d) mineral oil;
- e) perfume and colour.

P.2 Type 2

Based on vegetable oils and waxes.

- a) castor oil;
- b) beeswax;
- c) paraffin wax;
- d) coconut oil;
- e) perfume and colour.

P.3 Type 3

Based on mineral oils and fatty acids.

- a) stearic acid;
- b) mineral oil;
- c) perfume and colour.

P.4 Type 4

Based on mixture of mineral and vegetable oils, animal fats and waxes.

- a) coconut oil;
- b) mineral oil;
- c) beeswax;
- d) petroleum jelly;
- e) lanolin;
- f) perfume and colour

Annex Q
(normative)

Antibacterial test

Q.1 Procedure

Prepare nutrient agar for bacterial growth by dispersing 28 g of nutrient agar powder in 1 litre of deionized water. Allow to soak for 10 minutes, swirl to mix and then heat gently with stirring to ensure uniformity. Sterilize by autoclaving for 15 minutes at 121 °C, cool at 47 °C, mix well and then pour to sterilized petri dishes. Leave it to solidify undisturbed. Plant *Staphylococcus aureus*, *pseudomonas aeruginosa* and *candida albicans* on the so prepared nutrient agar in petri dishes.

Meanwhile, prepare filter paper discs and sterilize them by autoclaving. Dip in various samples. Place in the petri dishes containing bacteria culture agar mixture. Incubate the petri dishes at 35 °C for 48 hours. Determine bacteria growth inhibition zones.

Q.2 Antifungal test

Dissolve potato dextrose agar (39 g) in 1 dm³ of distilled water. Use the same procedure as for bacterial test (Q.1) above. Test the cream against fusarium fungi. Obtain the results after 4 days and the temperature of incubation shall be 25 °C.

Q.3 Results

The inhibition zone shall be at least 0.2 mm in diameter.

Annex R
(informative)

Available Kenya Standards on fats, oils and waxes

R.1 Mineral oils and waxes

- a) mineral oil - KS 03-796:1989¹⁾
- b) paraffin wax - KS 03-1426:1997²⁾
- c) microcrystalline wax - KS 03-1427:1997³⁾
- d) petroleum jelly - KS 03-581:1998⁴⁾

R.2 Vegetable oils

- a) sesame oil - KS 03 797:1988⁵⁾
- b) groundnut oil - KS 03-798:1989⁶⁾
- c) castor oil - KS 03-799:1989⁷⁾
- d) coconut oil - KS 03-800:1989⁸⁾
- e) mustard oil - expressed raw type
- f) avocado oil - KS 1765⁹⁾

R.3 Animal fats

- a) lanolin - KS 03-915:1989¹⁰⁾

¹⁾ Specification for mineral oil for cosmetic industry

²⁾ Specification for paraffin wax

³⁾ Specification for microcrystalline wax of petroleum origin

⁴⁾ Specification for pure petroleum jelly for human use

⁵⁾ Specification for sesame oil for petroleum industry

⁶⁾ Specification for groundnut/peanut oil for cosmetic industry

⁷⁾ Specification for castor oil for cosmetic industry

⁸⁾ Specification for coconut oil for cosmetic industry

⁹⁾ Specification for avocado oil for cosmetic industry

¹⁰⁾ Specification for anhydrous lanolin for cosmetic industry